Asian Journal of Innovative Research 3(2) 19-28 (2018)

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH

Available online at http://www.asianjir.com

Research article Botany

Received 11th Feb.2018;
Accepted 25 March. 2018
Online April 2018

SCREENING OF PHYTOCHEMICALS AND PHARMACOLOGICAL STUDIES ON *Mimosa pudica* L.

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ABSTRACT

In the present investigation was carried out the Screening of phytochemicals and pharmacological studies on *Mimosa pudica*. With regarding investigation to evaluate the biological activities of aqueous solvents of *Mimosa pudica* was investigated some of the phytochemicals like alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids were determind in qualitatively analysed whereas quantitatively the same bioactive compounds and a saponins, flavonoids, tannins and terpenoids with respective quality of 0.48, and 0.39 mg / ml estimated from *M. pudica* leaf extract. The antioxidant and anti-inflammatory activity proved. The antimicrobial acitivity of *Mimosa pudica* of different concentration of 25, 50, 75, and 100 mg was tested against *Bacillus cereus, E.coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* tested. The minimum zone of inhibition of 100mg concentration of *M.pudica* against *Pseudomonas aeruginosa* (15mm) observed respectively. The minimum zone of inhibition was *Bacillus cereus* (5mm) suppression observed in the bacterial sample respectively whereas antifungal activity also tested against *A.flavus, A.niger, A. terreus, Fusarium* sp and *Penicillium* sp. was tested among the fungi, the *Aspergillus terreus* was maximum zone of inhibition (17mm) from the test plant *M. pudica* and minimum inhibition (5mm) of *A. flavus* and *A.niger* from the plant extract the plant *M.pudica* was suitable for antimicrobial activity.

Keywords: Phytochemical, Antimicrobial, *Mimosa pudica*.

***Citation*:** G. Durgadevi and N. Karthika (2018). Screening of phytochemicals and pharmacological studies on *Mimosa pudica* L. *Asian Journal of Innovative Research* 3(2) 19-28 (2018)

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INTRODUCTION

Medicinal properties of plants are the most precious gift of Mother Nature to Mankind. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment. Various medicinal plants have been used for years in daily life to treat diseases all over the World. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness by Balakumar and Rajan (2011). The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment / management of various diseases. Herbal drugs or medicinal plants, their extracts and their isolated compounds have demonstrated spectrum of biological activities and continued to be used as medicine in folklore or food supplement for various disorders (Chauhan *et al.*, (2009). In the present study to investigate the screening of phytochemicals and pharmacological studies on *Mimosa pudica* L.

MATERIALS AND METHODS

Collection of plant material

The plant materials *Mimosa pudica* was collected from
the Thanjavur District, Tamil Nadu

Preparation of plant powder

The collected plant sample was air dried. After air dried the samples was ground in grinding machine made for the laboratory. Exposure direct sunlight and avoided to prevent the loss of active components. These powderedmaterials were used for further analysis.

Analysis of proximate content of *Mimosa pudica*

Determination of moisture: (James 1995). Determination of Crude Fiber (CF). Determination of nitrogen free extracts (NFE), Acid Soluble and Insoluble Ash and Determination of Ash

Preparation of Leaf extract

Fresh leaves of *M.pudica* were washed with sterilized water then crushed with festle and moter. Phytochemical test were carried out the aqueous extract and on the specimens were using standard procedures to indentify constituents are described by Sofowara (1993), Treas and Evans (1989) and Harborne (1973).

Qualitative phytochemical analysis

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) and Evans (1996).

Quantitative analysis

Alkaloid determination by using Harborne (1973) method. Estimation of flavonoids (Krishnah *et al.*,2009) Estimation of tannins (Van – Burden and Robinson, 1981).Estimation of total phenols. Determination of antimicrobial activity (Perez *et al.*, 1990)

Test microorganisms:

The following bacterial and fungal strains were used for the screening of antimicrobial activity. All the microbial strains of human pathogens used were procured from IMTECH, Chandigarh and procured microbes are the Gram – negative bacteria, viz. *Escherichia coli* , *Protease valgaris*, *Pseudomonas aeruginosa* and the Gram – positive bacteria, *Bacillus cerres* and *Staphylococcus aureus*,and fungi viz., *Aspergillus flavus*, *A.niger*, *A. terreus*, *Fusarium* sp, and *Penicillium* sp were selected for this study.

Media used:

Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used for testing the antibacterial and antifungal activity.

Agar well – diffusion method

Agar well – diffusion method was followed for determination of antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old – broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About 100µl of different solvent leaf extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre – incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions the plates were incubated in an upright position at 37˚C ± 2˚C for 24 h for bacterial pathogens and 28˚C ± 2˚C for fungi. The organic solvents alone were acted as a negative control. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. The effect of plant extract was compared with standard antibiotics. Triplicates were maintained and the average values were recorded for antimicrobial activity.

***In vitro* ANTI – INFLAMMATORY ACTIVITY**

Methods of Mizushima and Kobayashi (1968) followed with minor modifications.

ANTIOXIDENT ACTIVITY

DPPH Free radical scavenging activity (Yordabov and Christova, 1997). Thiobarbituric Acid (TBA) Method (Sawarka *et al.*, 2009). Scavenging activity of H_2O_2 radical activity (Ruch

et al., 1989) Reducing power (Oyaizu, 1986), Scavenging activity of superoxide Dismutase(*Yen and Chen* 1995)

RESULTS

The proximate analysis of *M.pudica* was analysed and presented in (Table – 1). The crude fiber content of *M.pudica* was maximum 3.30 % compared to their proximate tested similarly its lowered content of fat (0.01 %) was observed in its powered of *M.pudica*. The Moisture and Ash content of *M. pudica* were 0.15 % , Ash 0.06 % respectively (Fig - 1). The biological action of the plant extract can be ascertained only by the phytochemical reaction and this function was performed. The phytochemicals estimation such as alkaloids, flavnoids, phenol, phylobatannins, proteins, reducing sugar, saponins, steroids, tannins and terpenoids were dentified by qualitative indication. Among the ten test only that alkaloids, flavnoids, saponins, steroids, tannins and terpenoids was performed by method (Table -2).

Among to the quantitative phytochemical analysis of saponins, flavonoids, tannins and terpenoids were 0.48, 0.99, 0.80, 0.39 and 0.39mg/ml recorded respectively. The maximum of phenolic content which analysed from the *Mimosa pudica* with aqueous extract and minimum amount of terpenoids 0.39 mg/ml recorded (Table – 3).

The main aim of the free radical activity can be measured with various methods followed. The hydrogen peroxide severing method with *Mimosa pudica* extract of 0.2, 0.4, 0.6,0.8 and 1.0% on the effect antioxidant activity was 34.6, 39.4, 49.6, 54.6 and 58.3% recorded respectively. whereas reducing power assay with same concentration of *Mimosa pudica* with aqueous extract was 59.8, 64.5, 79.3, 89.8 and 94.7 % antioxidant activity observed respectively (Table – 4 and 5 Fig -2 and 3). The another method of thiobarbitaric acid of different concentration of 0.2, 0.4, 0.6, 0.8 and 1.0% of *Mimosa pudica* against the antioxidant activity of 59.7, 63.7, 69.1, 73.3 and 86.3% level performance recorded (Table -6 Fig - 4). The effect of anti-inflammatory activity of *Mimosa pudica* was higly responsible for reducing activity of inflammation when compared with diclofenae sodium and different concentration of *M. pudica* of 0.2, 0.6, 0.6, 0.8 and 1.0% with anti-inflammatory activity in 51.5, 59.7, 55.7, 71.5 and 83.7 % observed from the effect of *M .pudica* plant (Table -7 Fig - 5).

As per the egg methods of *Mimosa pudica* extract with different concentration of diclofenae sodium and 0.2, 0.4, 0.6, 0.8 and 1.0 % with anti-inflammatory properties of 42.5, 39.6, 48.2, 56.7, 65.3 and 76.7% recorded (Table -8, Fig - 6).

The whole plant products having medicinal plants of their properties are commonly known as medicinal plant. These medicinal plants of *Mimosa pudica* are known to posses various phytochemical which exhibit more bioactive such as antibacterial antifungal and anti-inflammatory activities were conformed from the investigation.

The effect of antibacterial activity *Mimosa pudica* against some bacteria were tested. The antibacterial properties of *M. pudica* against *Bacillus cereus* was 5, 6, 8 and 10 mm zone of inhibition 100 mg concentration of plant extract. Whereas *E.coli* was 9, 11, 15 and 22mm inhibition from the plant *M. pudica* extract. In the case of *Proteaus vulgaris* 14, 15 and 9mm with 25, 50, 75 and 100 mg concentration of plant extract tested. There is no zone of inhibition observed at 25 mg against *Proteaus vulgaris* bacteria. In the *Pseudomonas auroginosa* bacteria were 4, 6, 7, and 15mm zone of inhibition recorded with 25, 50, 75 and 100 mg concentration respectively. The special case of *Staphylococcus aureus* was 11, 13, 15, 18mm zone of inhibition from the concentration of 25, 50, 75 and 100mg of plant extract analysed respectively (Table-5) effect of antifungal activities of *M.pudica* with different concentration of 25, 50, 75 and 100mg against *Aspergillus flavus* was 5, 8, 9 and 12mm zone of inhibition measured. whereas *A.niger* 5, 6, 13 and 14 zone of measured and *A.terreus* was 8, 10, 11 and 17 mm and other fungi *Fusarium* sp. was 6, 7,10 and 15mm observed finally *Penicillum* sp. was 6, 8, 9 and 11mm zone of measured from the inhibition recorded respectively (Table- 9 and 10, Fig- 7 and 8).

DISCUSSION

In the recent research investigation suggestive that the *Mimosa pudica* plant was many more medicinal properties from almost in word wide application. In the study entitled on screening of phytochemical and pharmacological studies on *mimosa pudica* was observed. The plant derived compounds have an increasing interest throughout the word as they possess potent. About the word population is relying on traditional medicinals where the whole or plants is used as medicinal. Drug resistance in microorganisms has become a un solvable problem and treating an infection disease with the exciting drug is becoming less uses. The quantitative estimation of the percentage of phytochemicals contain in *Mimosa pudica* was performed. The whole plant consist of alkaloid 9.05 %, flavonoid 8.32 %, steroid 2.49 %, saponin 8.15 %, phenol 1.02 %, tannin 0.083 %, cyanogenic glycoside0.122 % and anthocyanin 1.913 %. Alkaloids are the most efficient phytochemical compound. True isolated alkaloids and the synthetic derivatives are used as the basic medicinal compounds because of their analgesic, anti-spasmodic and bacterial properties Salah *et al.*, (1998). The presence of tannins and phenols in the plant can attest to its use for healing of wounds, hemorrhoids in herbal medicine.

The preliminary phytochemical screening of ethanol extract showed the presence of steroids, carbohydrates, saponins, flavonoids and Tannins The results of the antimicrobial assay of the ethanol extract of *Mimosa pudica* indicated that the plant exhibited antimicrobial activity against the tested microorganisms at four different concentrations of 25 µl, 50 µl, 75 µl and 100 µl/disc. The potential sensitivity of the extract was obtained against all the microorganisms tested the zone of inhibition was recorded and presented by Chopra *et al.,* (1992).

Medicinal plants possess a variety of compounds of known therapeutic properties Ahmadet and Beg (2001) Hence, much attention has been paid to plant derived antibacterial compounds based on the knowledge that plants have their own defense system. The therapeutic use of such plant products is an alternative strategy to prevent the spread of disease. In the present investigation the active phytocomponents of *M.pudica* was studied and further the antimicrobial activity of the plant extract was also tested against potentially pathogenic microorganisms *B.subtilis, P.aeruginosa K.pneumoniae, A.flavus* and *T.rubrum* at different concentrations of the extract to understand the most effective activity. The maximum zone of inhibition was obtained for *B.subtilis* and *A. flavus* at a concentration of 100 µl. The minimum zone of inhibition was observed in all tested organisms at a concentration of 25µl.

The phytochemical analysis showed the presence of flavonoids, phenolic compounds, glycosides, alkaloids, carbohydrates and proteins in ethanolic and aqueous leaf extracts of *Prosopis juliflora* and also in *Mimosa pudica*. Steroids were present in the ethanolic leaf extracts of Prosopis juliflora and *Mimosa pudica*. But tannins and saponins were present only in the aqueous leaf extracts of *Prosopis juliflora* and *Mimosa pudica.* It is suggested strongly that the alkaloidal fraction isolated from *Prosopis juliflora* was found to possess significant antibacterial activity (Ahmad Aqeel, 1991). It is reported in a study that preliminary phytochemical screening of *Prosopis juliflora* leaves revealed the presence of tannins, glycosides, flavonoids and alkaloids (Sathiya and Muthuchelian, 2008). Also a study supports that *Prosopis juliflora* possesses an unusual amount of the flavonoid-mesquitol in its heartwood (Peter Sirmah *et al.,* 2009). Phytochemical analysis of the extracts revealed the presence of tannins, phenolics, flavonoids, alkaloids, terpenes and steroids in most parts of *P. juliflora* (Shachi Singh, 2012). It is revealed in a study that phytochemical screening of the *Prosopis juliflora* leaf extract showed the presence of alkaloids, flavonoids, steroids, phenolics and tannins (Senbagarani Renganathan *et al*, 2015).

The extracts of *M. pudica* leaves shows highest zone of inhibition against *Trichophytom verrucosum* and *T. soudanense* but not effective against other isolates. The presence of phytochemical compounds of the studied plants part, the inhibitory zone and concentrations at which values were effective on the tested organisms highlight that there were variations in the antifungal potency of the plants. The variation in sensitivity could also be attributed to differences in growth rate of isolated organism nutritional requirement, temperature and inoculums size Khalimuthu *et al.,* (2010). Similar were obtained by whose revealed maximum zone of inhibition when *M. pudica* leaf extracts were used against *E. coli, Lactobacillus* and *Salmonella typhi.*

The extracts indicate significant difference inhibitory activity of aqueous and ethanol extracts. Antifungal activity of the ethanol extracts appeared to be more effective than aqueous extracts since ethanol could extract a wide variety of active components as compared to aqueous Kaur *et al.,* (2011).

The medicinal herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel, 1991). Although a significant number of studies have been used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veermuthu *et al*., 2006). In the present investigation, the active phytocomponents of *M. pudica* was studied and further the antimicrobial activity of the plant extract was also tested against three potentially pathogenic micro-organisms *S. aureus, E. coli,P. aeruginosa,* Pyrogen and *C. albicans* at different concentrations of the extract to understand the most effective activity. The maximum zone of inhibition was obtained for*E. coli* and *S. aureus* at a concentration of 35 mg/ml. While *P. aeruginosa* exhibited good sensitivity against both the concentrations. From the studies, it is concluded that the traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethno-medical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

The quantitative estimation of the percentage yields of chemical constituents of the plants studied showed that they are rich in protein with *B.pilosa* having the highest protein content.

M. pudica had the lowest protein level in all the plants investigated and could be administered to patients that does not need much protein. *M. pudica* showed a high fat level out of the plants studied and explains why it may not be useful for those having high fat content already. Epidemological evidences have shown that consumption of reasonable amount of dietary fibre (20 – 35g/day) lower risk of a number of chronic diet related diseases such as diverticular disease, coronary heart disease, obesity, type 2 diabetes mellitus, irritable bowel syndrome, etc., Houghton (2007). *E. hirta and C. zambesicus* are best prescribed for diabetic patients because of their low fat level. *L inarmic, M. pudica* and *P. vulgaris* had high ash content and makes them suitable as source of minerals and agrees with the findings of *P. vulgaris* had the highest crude fibre with *E.hirta* having the lowest crude fibre among the plants investigated. *P. americana* had the highest carbohydrate content and *B. vulgaris* with the lowest carbohydrate this makes the its extract of this plant good energy source.

The phytochemicals have many ecological and physiological roles as widely distributed plant constituents. Phytochemicals exhibit wide range of biological effects as constituents with their own antioxidant properties. The phytochemical analysis of the extract indicated the presence of 11 compounds. The leaves showed a number of phytoconstituents in methanol extract. In a previous study, Tamilarasi and Ananthi (2012) has reported the presence of 7 compounds in ethanol extract. Similar results were observed in this study also. These compounds are known to be biologically active and therefore aid the antimicrobial activity. The alkaloid has strong anticancer properties; Tannins have been found to form irreversible complexes with highly rich protein resulting in the inhibition of cell protein synthesis. They are known to react with protein to provide difficult tanning effect which is important for the treatment of influenced or ulcerated tissues. Herbs that that have tannins as the main component have astringent activity and are also used for treating intestinal disorder such as diarrhea and dysentery. The presence of tannin in *Mimosa pudica* is exploited in the traditional treatment for ailments (Savithramma *et al.*, 2011).

In the present investigation suggested that the phytochemical analysis of *Mimosa pudica* plant has alkaloid, flavonoids, phenol, phlobatannins, protein, reducing sugar, saponins, steroids, tannins and terpeniods recorded respectively. Whereas quantitatively saponins, flavonoids, tannins and terpenoids were showed that maximum extract from the *Mimosa pudica* plants.

In the recent research stated that the antioxidant activity was carried out by the reducing power assay has excellent activity posses when compared with other method of hydrogen proxide scavening assay and thiobarbataine assay. In the case of antioxidant activity of *Mimosa pudica* with different concentration treated from 0.2 to 1.0 mg but the results shown that 94.7% observed respectively. The medicinal plant *M. pudica* has remarkable bioactive compounds from the investigation

On the other hand, the excellent antioxidant activity but inflammatory properties are also reliable changing of bioactive molecules were observed and recorded respectively. In the aspects the plant *M. pudica* almost 77% of anti-inflammatory activity showed with *invitro* investigation and reliable natural properties also observed from the study, from the analysis, it has been confirmed that aqueous extract of *M.pudica* are potent biological properties observed.

About the antimicrobial activity of *M pudica* has maximum potential for *Pseudomonas auroginosa* bacteria with 75mg concentration of plant than followed by *Proteus vulgaris* (15mm) observed respectively and another study of maximum antifungal potential of *M. pudica* has *Aspergillus terreus* (17mm) zone of inhibition observed than followed by *A.niger* (14mm) at 100mg concentration of plant extract observed. The minimum zone of inhibition at (each 5mm) on *Aspergillus flavus* of 25 and 50 mg and another fungi *Fusarium* sp. (5mm) were measured from the 100mg concentration of plant extract of *Mimosa pudica.*

CONCULSION

From its present study it is concluded that *M.pudica* is the best Source of some of the bioactivity compounds which is used to some Pharmacological activity. Further study to be needed to extract the various phytochemical and its Biological activity of *M.pudica.*

Table 1: Analysis of proximate content of *Mimosa pudica*

S. No	proximate content	Quantity (%)
1	Moisture	0.15
2	Ash	0.06
4	Fat	0.01
5	Crude Fibre	3.30

Table 2: Qualitative analysis of phytochemical compounds of *Mimosa pudica*

Phytochemical compounds	Aqueous
Alkaloids	+
Flavonoids	+
Phenol	-
Phlobatannins	-
Protein	+
Reducing sugar	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

(+) – present - (-) absent

Table 3: Quantative analysis of phytochemical compounds of *Mimosa pudica*

phytochemical compounds	Quantity (mg/ml)
Saponins	0.48
Flavonoids	0.99
Tannins	0.80
Terpenoids	0.39

Table 4: Antioxidant activity of *M. pudica* plant by Hydrogen peroxide scavenging (H_2O_2) assay

Mimosa pudica extract (%)	(%) activity
0.2	34.6
0.4	39.4
0.6	49.6
0.8	54.6
1.0	58.3

Table 5: Antioxidant activity of *M. pudica* plant by Reducing power assay

Mimosa pudica extract (%)	(%) activity
0.2	59.8
0.4	64.5
0.6	79.3
0.8	89.8
1.0	94.7

Table 6: Antioxidant activity of *M. pudica* plant by Thiobarbitaric Acid.

Mimosa pudica extract (%)	(%) activity
0.2	59.7
0.4	63.7
0.6	69.1
0.8	73.3
1.0	86.3

Table 7*: In vitro* anti inflammatory effect of *Mimosa pudica* Bovine:serum albumin

***Mimosa pudica* extract (%)**	**(%) activity**
Diclofenac sodium	51.5
0.2	59.7
0.4	55.7
0.6	71.5
0.8	75.7
1.0	83.7

Table 8*: In vitro* anti inflammatory effect of *Mimosa pudica* Egg:

***Mimosa pudica* extract (%)**	**(%) activity**
Diclofenac sodium	42.5
0.2	39.6
0.4	48.2
0.6	56.7
0.8	65.3
1.0	76.7

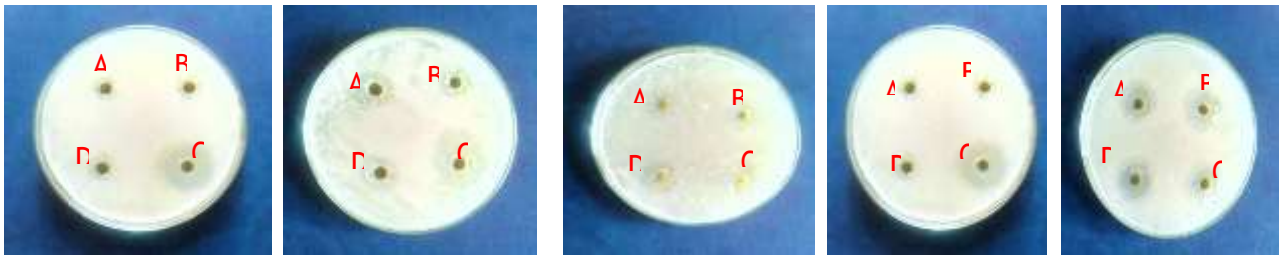
Table 9 : Studies on the effect of antibacterial activity of *Mimosa pudica* against bacteria

S.no	**Name of bacteria**	**Zone of inhibition (mm)**			
		25 mg	**50 mg**	**75 mg**	**100 mg**
1	*Bacillus cereus*	5	6	8	10
2	*E. coli*	9	11	15	22
3	*Proteus valgaris*	-	14	-	9
4	*Pseudomonas auroginosa*	4	6	7	15
5	*Staphlococcus aureus*	11	13	15	18

Table 10 : Studies on the effect of anti fungal activity of *Mimosa pudica* against fungi

S.no	**Name of fungi**	**Zone of inhibition(mm)**			
		25 mg	**50 mg**	**75 mg**	**100 mg**
1	*Aspersillus flavus*	5	8	9	12
2	*A.niger*	5	6	13	14
3	*A. terreus*	8	10	11	17
4	*Fusarium sp.*	6	7	10	15
5	*Penicillium* sp.	6	8	9	11

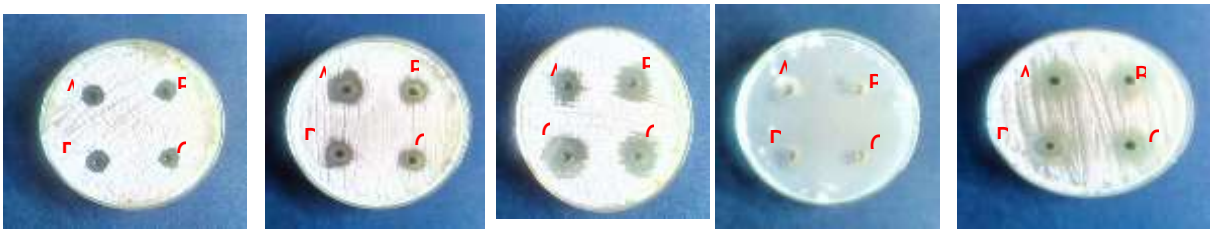
Plate 3: Studies on the effect of antibacterial activity of *Mimosa pudica* against bacteria



Bacillus cereus *E. coli* *Proteus valgaris* *Pseudomonas auroginosa* *Staph.aureus*

A – 25 mg, B – 50 mg, C – 75 mg and D – 100 mg

Plate 4 : Studies on the effect of anti fungal activity of *Mimosa pudica* against fungi



Aspersillus flavus *A.niger* *A.terreus* *Fusarium* sp. *Penicillum* sp

A – 25 mg, B – 50 mg, C – 75 mg and D – 100 mg

Fig 1: Analysis of proximate content of *Mimosa pudica* plant

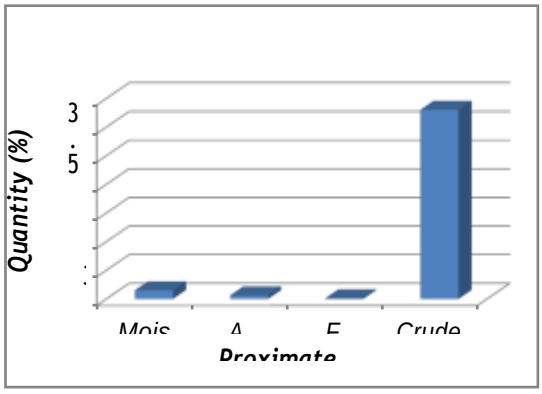


Fig 2: Antioxidant activity of *Mimosa pudica*

Hydrogen peroxide scavenging (H_2O_2) assay.

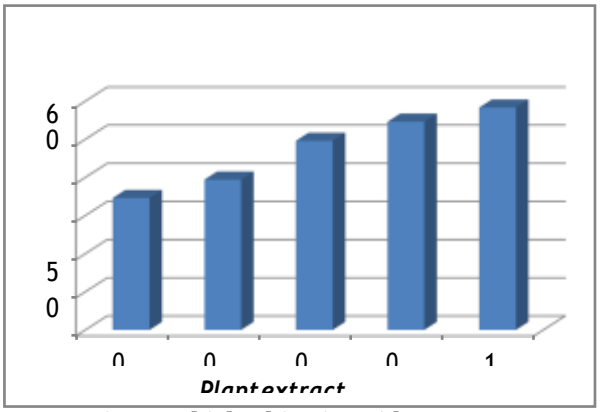
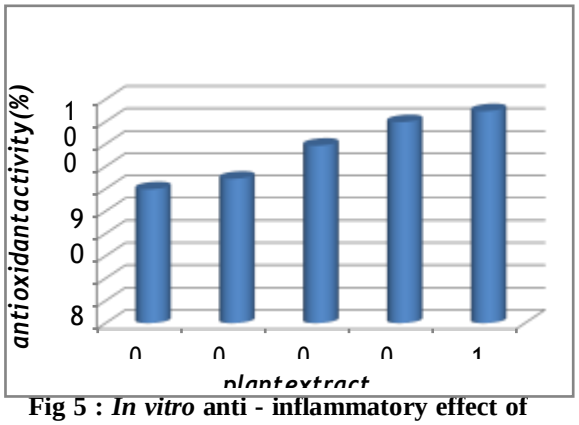


Fig 3: Reducing power assay

Fig 4: . Thiobarbitaric Acid



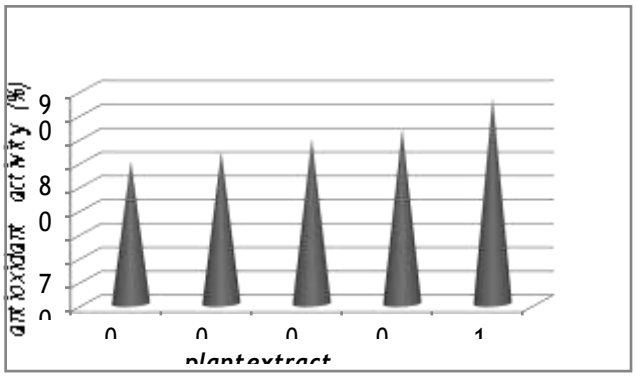
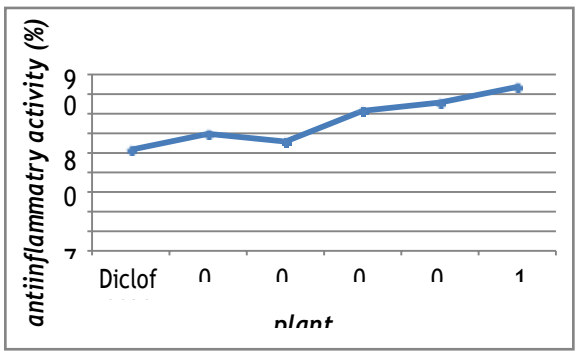


Fig 5 : *In vitro* anti - inflammatory effect of *Mimosa pudica* by bovine: serum albumin

Fig 6: *In vitro* anti - inflammatory effect of *Mimosa pudica* by Egg:



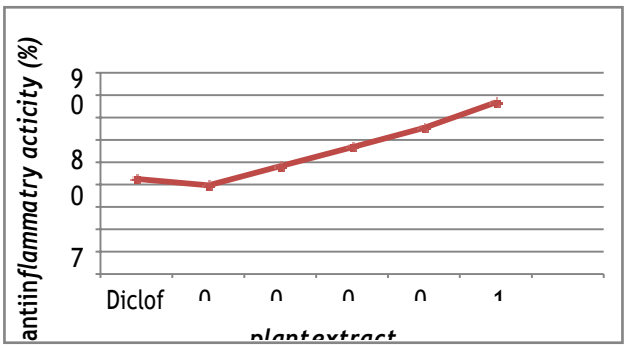


Fig 7: Studies on the effect of antibacterial activity of *Mimosa pudica* against bacteria

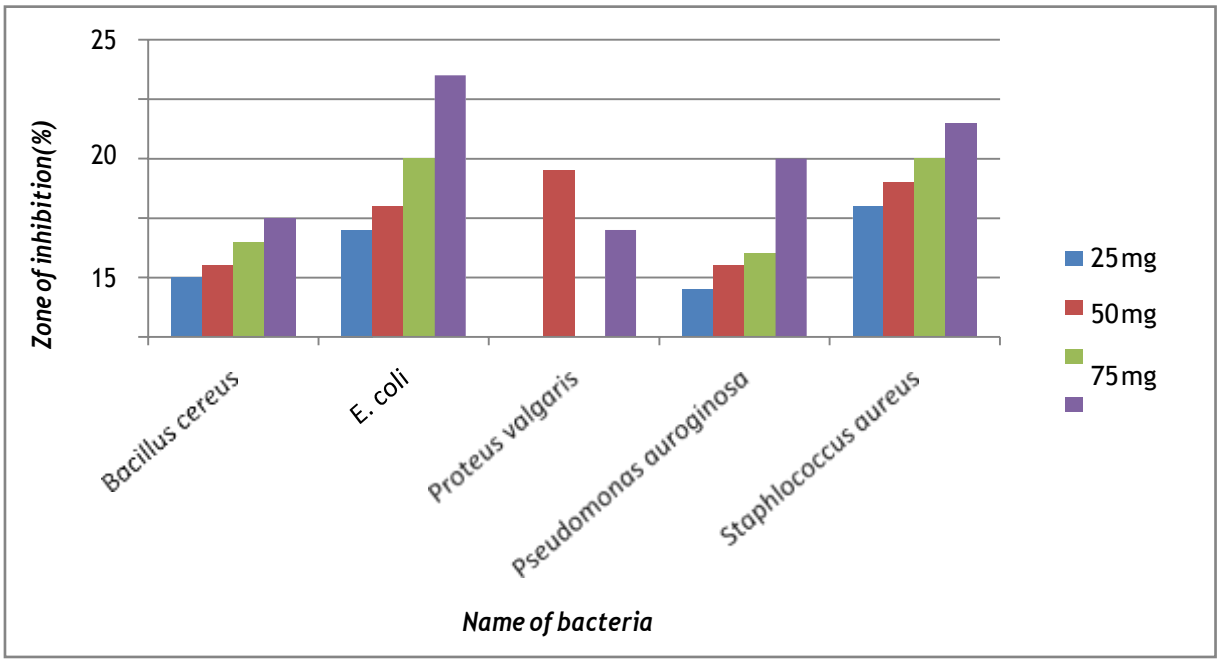
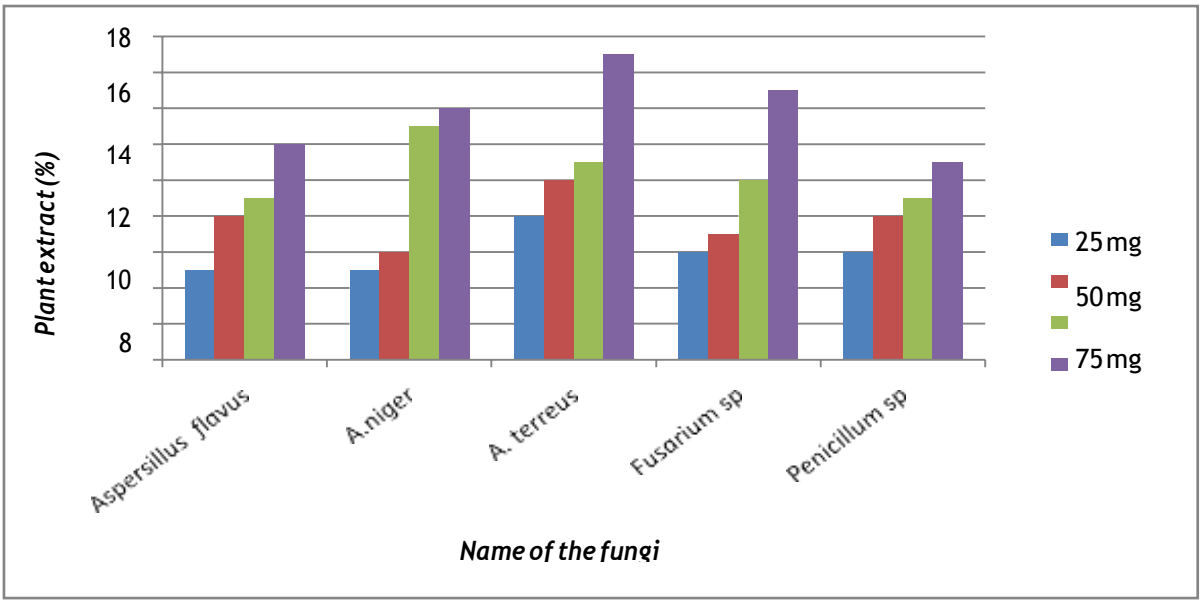


Fig 8: Studies on the effect of anti fungal activity of *Mimosa pudica* against fungi



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Source of support: Nil;
Conflict of interest: None declared